The emergence and spread of dysentery

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***Shigella sonnei* is an important cause of bacterial dysentery in the developed world, and has also recently emerged in transitional countries. Phylogenetic analysis based on whole-genome sequencing of a global sample has detailed the recent evolutionary history of this pathogen, and shed light on the genetic changes associated with this epidemiological shift.**

Shigella are human associated pathogenic *E. coli*, transmitted by the fecal-oral route, and are responsible for at least 120 million cases of dysentery each year, with approximately 1 million fatalities[1]. On page xx of this issue, Nicholas Thomson and colleagues[2] describe a detailed evolutionary analysis of a diverse collection of *Shigella sonnei isolates.* The work provides an exemplar of how next-generation sequencing has transformed our ability to reconstruct historical and contemporary patterns of transmission and genome divergence within bacterial pathogens[3]

Four *Shigella* serogroups have been described, each of which independently acquired a pINV plasmid conferring the ability to invade the gut mucosa[4]. These serogroups exhibit distinct epidemiological properties; for example, *S. flexneri* is waterborne and prevalent in the developing world, whereas *S. sonnei*, which is less diverse than other serogroups, more typically causes disease in the developed world via person-to-person spread or contaminated food[5]. Improved sanitation over last few decades has led to a decrease in *S. flexneri* cases in the developing world, but has coincided with the emergence of *S. sonnei*.

Sequencing and phylogenetic analysis

Holt et al. assembled 132 *S. sonnei* strains isolated between 1943 and 2008 from four continents. This diverse sample allowed the inference of long-range transmission events and local expansion, while the inclusion of older isolates enabled reliable temporal calibration of the tree. The whole genomes of pools of 11 or 12 isolates were sequenced on the Illumina Genome Analyzer GAII, and the authors reconstructed robust maximum likelihood trees using RAxML with *E. coli* and *Shigella* outgroups. Given the tree topology and isolation dates it is possible to test for temporal signal in the data, and to date the major nodes in the tree. Holt et al. estimated a mean mutation rate of 6×10^{-7} site^{-1} year^{-1}, approximately half the rate of *S. aureus* [6], but over twice as fast as the highly monomorphic *Yersinia pestis*[7]. This rate variation between taxa is most readily explained by longer generation times in more slowly evolving species.

Holt et al. estimate the most recent common ancestor of *S. sonnei* to have emerged under 500 years ago, far more recently than previously thought[8]. Four robust lineages are resolved from the data, one of which (lineage IV) is represented by a single isolate. These major divisions are consistent with *S. sonnei* biotypes , CRISPR

types.and are likely to be resolved by IST, MLVA[9] and PFGE, as these methods are broadly consistent with each other [10]. The authors used BEAST to infer that lineage I and II emerged in the early 19th century, while lineage III is dated to the late 19th century. Isolates from Europe are dispersed throughout the tree, and different methods of analysis provided consistent evidence for a European origin for *S. sonnei* as whole, as well as for each of the individual lineages (Figure 1).

The stability of these four *S. sonnei* lineages points to ecological or epidemiological differences. Regression analysis of root-to-tip distance against isolation date suggests that lineage III mutates more quickly than I and II, which the authors claim might be owing to population level effects consistent with different niches. More direct evidence is provided by the non-random distribution of the non-European isolates between the different lineages. Isolates from Asia, Africa and America are largely restricted to lineage III and in particular are rarely found in lineage I. The implication is that lineage III isolates are more capable of establishing local populations subsequent to inter-continental transmission. Moreover, one particular lineage III cluster (named Global III), which emerged in the early 1970s, corresponds to almost half the isolates in the sample recovered after 1995, and is widely geographically distributed. This cluster contains even finer-scaled clusters associated with particular countries, pointing to local clonal expansion. The inter-continental transmission events out of Europe inferred by the authors are summarised in Figure 1.

Spread and antibiotic resistance

Holt et al examined the distribution of known *S. sonnei* antimicrobial resistance mutations within the phylogeny, and found that global dissemination is strongly associated with multiple drug resistance (MDR). The independent acquisition of mutations in the same resistance gene within different clusters suggests strong selective pressure. Although antimicrobial treatment has little effect on *Shigella*, Holt et al speculate that the advantage conferred by resistance may operate by increasing shedding (and transmissibility) of the bacteria[11], rather than simply enhanced survival in the host. The acquisition of Class 2 integrons in biotype g (lineage III) has been implicated in increased spread of *S. sonnei*, and is known to have occurred in southern Italy by the late 1980s[12]. The data of Holt et al, reveal three independent acquisitions of these elements within lineage III isolates during the 1960s and 1970s, immediately prior to international dissemination. It remains unclear why class 2 integrons are not found in other lineages, one possibility being the presence of potentiating mutations specifc to lineage III.

The key role of antibiotic resistance genes in the global dissemination of specific *S. sonnei* clusters is underscored by limited evidence for positive selection in other genes. However, the analysis excludes the 180Kb pINV B plasmid, key in *S. sonnei* pathogenesis. This plasmid harbours the locus encoding O antigen biosynthesis, known to be under intense selection, and horizontally acquired by *S. sonnei* from *Plesiomonas shigelloides*[13], a gastroenteritis causing Enterobacteria associated with poor water quality. Holt et al. argue that the cross-reactivity in O antigens between *S. sonnei* and *P. shigelloides* helps to explain the recent emergence of *S. sonnei* in transitional countries as improved sanitation would reduce

environmental immunisation induced by *P. shigelloides* infection[14]. Although potentially a public health conundrum, the benefits of clean drinking water will likely be of greater significance.

Next steps

Holt et al. neatly illustrate how whole-genome sequencing of pathogens combined with phylogenetic analysis can be used to draw inferences over multiple scales, and that a small number of recently emerged clones are responsible for a disproportionately large share of the global public health burden resulting from *S. sonnei* infection. The acquisition of antibiotic resistance plays a key role in the spread of these clones, although the precise selective advantage conferred is unclear. To what degree the decline in environmental *P. shigelloides* has impacted on the spread of *S. sonnei*, and how this might possibly dovetail with the acquisition of antibiotic resistance, is also unknown. Finally, although Holt et al. raise the relevance for development of a vaccine, the implications for outbreak investigation are perhaps even more pertinent. Intercontinental travel and mass gatherings sharing a common food source[15] present ongoing risks for *S. sonnei* infection, and currently PFGE is the standard tool for epidemiological investigation.

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